Apigenin (mainly from parsley)

Summary:
-strong anti-oxidant, anti-inflammatory
- but still used as co-treatment with chemodrugs
- still can increase ROS (blocked the pretreatment of NAC anti-oxidant)
- difficult to eat enough (1–5μmol/L blood plasma level), need lots of supplement. Perhaps Liposomal Apigenin or nanocarriers? (take with sunflower lecithin?)
"4g of dried parsley will be enough for 50kg adult"
5mg/kg BW yields 16uM, so 80Kg person means 400mg (if dried parsley is 130mg/g, then would need 3g/d)
-note half-life is 2.5 hrs so might be better to spread out consumption thru the day.

Pathways:
- mediated by excessive ROS and ATP depletion
- multiple signaling pathways, including PI3K/AKT, NF-kB, JAK/STATs, Wnt/β-catenin, AMPK, MAPK/ERK, and JNK.
- downregulation of PI3K/Akt and NF-κB and CK2α expression
- GLUT-1, HIF-1α, and VEGF mRNA transcription and protein production both are suppressed by apigenin
- MCT1 inhibitor?
- shown to modulate the nuclear translocation of SREBP-2 (related to cholesterol)
- downregulate catalase and GSH
- conflicting evidence if Api increases or diminishes the expression of Nrf2 (most evidence says increases Nrf2) (diminish would be better for pro-oxidant approach) **** Parsley also contains Luteolin which is an Nrf2 inhibtor
-combined with Metformin(reduces Nrf2) amplifies ROS production
- ROS effect of Api blocked by : antioxidant N-acetylcysteine, p53 inhibitor pifithrin-α, and enzyme catalase

https://www.mcsformulas.com/vitamins-supplements/apigenin-pro-liposomal/ Apigenin Pro Liposomal, 200 mg (has sunflower lecithin)

https://pubmed.ncbi.nlm.nih.gov/29034071/

Apigenin in cancer therapy: anti-cancer effects and mechanisms of action 2017

Apigenin is a common dietary flavonoid that is abundantly present in many fruits, vegetables and Chinese medicinal herbs and serves multiple physiological functions, such as strong anti-inflammatory, antioxidant, antibacterial and antiviral activities and blood pressure reduction. Therefore, apigenin has been used as a traditional medicine for centuries. Recently, apigenin has been widely investigated for its anti-cancer activities and low toxicity. Apigenin was reported to suppress various human cancers in vitro and in vivo by multiple biological effects, such as triggering cell apoptosis and autophagy, inducing cell cycle arrest, suppressing cell migration and invasion, and stimulating an immune response. In this review, we focus on the most recent advances in the anti-cancer effects of apigenin and their underlying mechanisms, and we summarize the signaling pathways modulated by apigenin, including the PI3K/AKT, MAPK/ERK, JAK/STAT, NF-κB and Wnt/β-catenin pathways. We also discuss combinatorial strategies to enhance the anti-cancer effect of apigenin on various cancers and its use as an adjuvant chemotherapeutic agent to overcome cancer drug resistance or to alleviate other adverse effects of chemotherapy. The functions of apigenin against cancer stem cells are also summarized and discussed. These data demonstrate that apigenin is a promising reagent for cancer therapy. Apigenin appears to have the potential to be developed either as a dietary supplement or as an adjuvant chemotherapeutic agent for cancer therapy.
And in human papillary thyroid carcinoma BCPAP cells, apigenin treatment caused G2/M cell cycle arrest via down-regulation of Cdc25c expression and stimulated the accumulation of reactive oxygen species (ROS) production, leading to induction of DNA damage [32].
Meanwhile, apigenin treatment significantly downregulated stemness marker Oct3/4 protein expression by downregulation of PI3K/Akt and NF-κB signaling pathways. [65].
Apigenin was demonstrated to downregulate CK2α expression and inhibited the self-renewal capacity of SFCs in SKOV3 and HeLa cells [66, 67]. Meanwhile, by targeting CK2, apigenin synergistically enhanced PI3K/AKT inhibitor-induced apoptosis in CD34(+)CD38(−) leukemia cells without harming healthy hematopoietic stem cells [68].
Apigenin has been shown to inhibit AKT function in different cancer cell types by directly suppressing PI3K activity by blocking the ATP-binding site of PI3K and subsequently inhibiting AKT kinase activity [73].
In addition, apigenin inhibited the human PI3K/AKT/FOXO signaling pathway in human prostate cancer resulting in cell cycle arrest and cell apoptosis [77].
Apigenin has the potential to decrease GLUT-1 expression levels via downregulation of the PI3K/AKT signaling pathway in vitro and in vivo, which enhances xenograft radiosensitivity and inhibits tumor growth [78].
The Nuclear Factor-κappa B (NF-κB) signaling pathway is generally considered an active factor in survival and proliferation.
In most cases, apigenin treatment inhibits NF-κB activation both in vitro and in vivo.
Wnt/β-catenin signaling is highly conserved from sponge to human and plays important roles in metazoan development and tissue homeostasis.
The increased expression of Wnt, frizzle or lymphoid enhancer factor (LEF)/T cell factor (TCF) in this signaling pathway is commonly detected in patients with leukemia, colorectal cancer, breast cancer or adrenocortical tumors [103–106]. I
Apigenin was found to significantly inhibit the Wnt/β-catenin signaling pathway, thereby suppressing cell proliferation, migration, and invasion in colorectal and osteosarcoma cancers [11, 108].
Apigenin is an effective anti-cancer agent but with only moderate anti-cancer efficacy when used alone at human physiological dosages [24, 38, 113]. Therefore, co-treatment with other chemodrugs is a reasonable way to enhance its anti-cancer activities. The combination of apigenin and other chemodrugs are summarized in Table 2. In addition, most of the combination treatments resulted in enhanced anti-cancer efficacy in vitro and in vivo.
IFNγ ,Paclitaxel, Cisplatin, 5-Fluorouracil (5-FU), Doxorubicin and etoposide, ABT-263, miR-433-5p knockdown,
Apigenin shows antitumor activities by modulating multiple signaling pathways, including PI3K/AKT, NF-kB, JAK/STATs, Wnt/β-catenin, AMPK, MAPK/ERK, and JNK. We need to note that although so many signaling pathways are reported to be modulated by apigenin, it is still not clear whether there is cross regulation among those signaling pathways.

https://pmc.ncbi.nlm.nih.gov/articles/PMC7585594/

Apigenin causes necroptosis by inducing ROS accumulation, mitochondrial dysfunction, and ATP depletion in malignant mesothelioma cells 2020

Apigenin, a naturally occurring flavonoid, is known to exhibit significant anticancer activity.
Apigenin significantly inhibited cell viability with a concomitant increase in intracellular reactive oxygen species (ROS) and caused the loss of mitochondrial membrane potential (ΔΨm), and ATP depletion, resulting in apoptosis and necroptosis in monolayer cell culture.
Western blot analysis showed that apigenin treatment upregulated protein levels of cleaved caspase-3, cleaved PARP, p-MLKL, and p-RIP3 along with an increased Bax/Bcl-2 ratio.
However, pretreatment with ROS scavenger N-acetylcysteine (NAC) restored ATP levels reduced by apigenin. ATP supplementation improved cell viability (Fig.

3D) and recovered DDR-, apoptosis-, and necroptosis-inducing molecules upregulated by apigenin to near-basal levels (Fig. 3E). These findings indicated that apigenin-induced cell death was caused by ATP depletion, at least in part.
In conclusion, this study showed that apigenin exert a cytotoxic effect on human MM MSTO-211H and H-2452 cells by inducing necroptosis in an acidic environment. This effect of apigenin targets the mitochondria and is mediated by excessive ROS and ATP depletion. These results suggest that apigenin might have potential as a new anti-cancer agent.

https://pmc.ncbi.nlm.nih.gov/articles/PMC7593821/
Apigenin as Tumor Suppressor in Cancers: Biotherapeutic Activity, Nanodelivery, and Mechanisms With Emphasis on Pancreatic Cancer 2020
It seems that apigenin is capable of suppressing the proliferation of cancer cells *via* the induction of cell cycle arrest and apoptosis. Besides, apigenin inhibits metastasis via down-regulation of matrix metalloproteinases and the Akt signaling pathway. In pancreatic cancer cells, apigenin sensitizes cells in chemotherapy, and affects molecular pathways such as the hypoxia inducible factor (HIF), vascular endothelial growth factor (VEGF), and glucose transporter-1 (GLUT-1).
An increase in the expression level of HIF-1α is revealed in several cancers which is a contributory factor for drug resistance and higher mortality (Birner et al., 2001; Koukourakis et al., 2006).
The up-regulation of the glucose transporter (GLUT-1) is directly proportional to the poor prognosis in several cancers including ovarian, gastric, breast, and colorectal carcinomas (Zambrano et al., 2019).
It was revealed that glucose transporter GLUT-1 is blocked by apigenin (0–100 μM) under normoxic conditions (Melstrom et al., 2008).
Hypoxia-induced up-regulation of these three proteins is inhibited by apigenin (50 μM). Furthermore, apigenin obstructed the expression of the GLUT-1 and VEGF mRNA under hypoxia conditions in the mentioned cell lines. In normoxic and hypoxic conditions, GLUT-1, HIF-1α, and VEGF mRNA transcription and protein production both are suppressed by apigenin. This suggests apigenin as a potential anti-cancer drug for the treatment of the PC (Melstrom et al., 2011).
In suppressing the proliferation of cancer cells, apigenin can induce apoptotic cell death *via* increasing ROS generation, the down-regulation of anti-apoptotic factors Bcl-2 and Bcl-xl as well as the up-regulation of apoptotic factors Bax and Bim. Besides, apigenin can induce cell cycle arrest at the G2/M and S phases. In suppressing metastasis of cancer cells, apigenin administration interferes with the PI3K/Akt/mTOR signaling pathway as well as the expression of MMP-9, as a factor involved in the progression and invasion of cancer cells.
Despite its importance and useful effects, there is not enough literature on apigenin's beneficial health potential for humans. A good reason may be low solubility of apigenin in water (1.35 μg/mL) and its high permeability (Zhang et al., 2012). These may hamper the *in vivo* studies into apigenin. There are various strategies suggested to increase solubility, such as several delivery systems (nanosuspension, polymeric micelles, liposomes).

https://pmc.ncbi.nlm.nih.gov/articles/PMC8167032/
Does Oral Apigenin Have Real Potential for a Therapeutic Effect in the Context of Human Gastrointestinal and Other Cancers? 2021
Apigenin taken orally is systemically absorbed and recirculated by enterohepatic and local intestinal pathways. Its bioavailability is in the region of 30%. Once absorbed from the oral route it reaches maximal circulating concentration (C_{max}) after a time (T_{max}) of 0.5–2.5h, with an elimination half-life ($T^1/_2$) averaging 2.52 ± 0.56h.
Using a circulating concentration for efficacy of 1–5μmol/L as the target, we evaluated data from both human and rodent pharmacokinetic studies to determine if a therapeutic concentration would be feasible. We find that oral intake of dietary materials would require heroic ingestion amounts and is not feasible. However, use of supplements of semi-purified apigenin in capsule form could reach target blood levels using amounts that are within the range currently acceptable for other supplements and medications.
Flavonoids as a sub-class are poorly bioavailable from the diet due to their low water solubility, chemical instability, and rapid metabolism in the body (Leonarduzzi et al., 2009). Apigenin itself is however lipophilic and a weak acid, and therefore will be most permeable to cell membranes in the unionized form within acidic environments, allowing it to be better absorbed in lower pH environments along the gastrointestinal tract, including the stomach (Leonarduzzi et al., 2009). In the pH range of the intestinal tract, hydrophobic apigenin is still able to permeate lipid membranes–enabling absorption along the full length of the intestines, but does so most effectively in the duodenum (Tang et al., 2017).
Blood and urine samples from 11 German adults (ages 23–41) were taken following a meal consisting of 2g parsley/kg body weight–which was equivalent to ∼17mg of apigenin (Meyer et al., 2006). Plasma concentrations of apigenin ranged from 28–337nmol/L at 6–10h after consumption, and fell below detection at 28h (Meyer et al., 2006).
Parsley leaf itself is available in capsule form as a health supplement, with the typical recommended ingestion amount being 2 capsules for a total dose of 900mg. Using an apigenin content for dried parsley of 45mg/g (Sung et al., 2016), this would equate to a daily dose of approximately 40mg apigenin, less than 1% of what is needed for a meaningful clinical outcome on cancer cell behavior, although it may have other beneficial effects on health.
Use of apigenin supplements, with purified apigenin in capsules, can achieve biologically relevant plasma concentrations that would be capable of influencing cellular behaviors.

https://www.cancertreatmentsresearch.com/reduce-cholesterol-in-cancer-cells-to-fight-cancer/?highlight=apigenin
Update: 10-April-2017: when ACLYL is inhibited by HCA, tumor cells will express ACSS2 enzyme to convert acetate into the actyl-CoA (Ref.). ACSS2 level is modulated by SREBP2 (Ref.) and SREBP2 in turn can be modulated by Vitamin E derivatives (**tocotrienols**) (Ref.). **Apigenin** also attenuates SREBP-2 (Ref.).
According to malypaet (a contributor to this website) a spoon of fresh parsel by day would contain enough Apigenin to achieve the attenuation? (Ref.). Apigenin can also be found online as a supplement. ACLYL may also be inhibited by Omeprazole (Ref.).

https://www.cancertreatmentsresearch.com/reduce-cholesterol-in-cancer-cells-to-fight-cancer/?highlight=apigenin
I came today around article about apigenin – it was glass and mouse tested alone and showed anti-cancer effect on chondrosarcoma cells.
http://www.ijcem.com/files/ijcem0057902.pdf
“Apigenin inhibits proliferation of human chondrosarcoma cells via cell cycle arrest and mitochondrial apoptosis induced by ROS generation-an in vitro and in vivo study”
2 and 5 mg/kg dose was used.
According to https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4942635/
“Fresh parsley leaves (P. crispum) were purchased from a local store (Lodz, Poland) and dried at 150°C for 30 min prior to use.”
Petroselinum crispum 126 – 137 mg/g
So, 4g of dried parsley will be enough for 50kg adult.
If it is pro-oxidant then could it be combined with chemo (if drugs interactions is clear)?

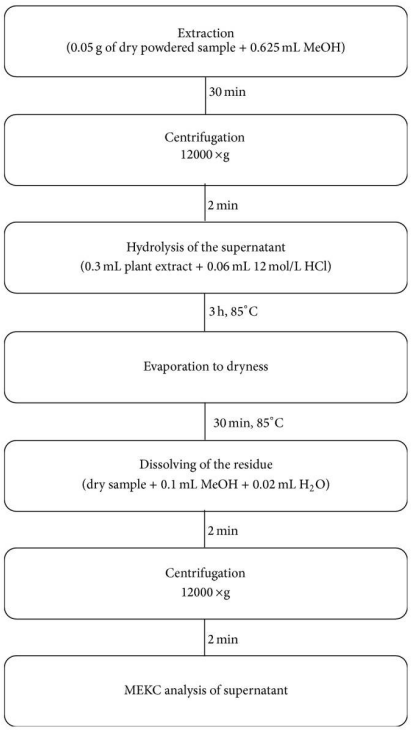
https://pmc.ncbi.nlm.nih.gov/articles/PMC4942635/
Determination of Total Apigenin in Herbs by Micellar Electrokinetic Chromatography with UV Detection 2016

In living organisms apigenin (API), a naturally occurring plant flavone, acts as antioxidant and exhibits anti-inflammatory activities and prevents LDL oxidation as well as oxidation of vitamins C and E and glutathione [6]. API (Figure 1) possesses also antimutagenic and antiviral properties and inhibits the proliferation of various human cancer cells, including breast, cervical, lung, liver, prostate, gastric, and hematologic cancer cells [7, 8].
Dry samples of *Petroselinum crispum*, *Rosmarinus L.*, *Thymus vulgaris L.*, *Origanum vulgare*, *Origanum majorana L.*, *Salvia officinalis L.*, and *Levisticum officinale* were purchased from the local supermarkets. Fresh parsley leaves (*P. crispum*) were purchased from a local store (Lodz, Poland) and dried at 150°C for 30 min prior to use.

Concentration of apigenin in different herbs.

Herb	Average value ± SD	
	(μmol/mL)	(mg/g)
Petroselinum crispum	122.4 ± 13.4[a]	137.7 ± 15.1[a]
	112.0 ± 12.2[b]	126.0 ± 13.7[b]
Rosmarinus L.	0.5 ± 0.2[c]	0.5 ± 0.2[c]
Thymus vulgaris L.	0.7 ± 0.3[c]	0.7 ± 0.3[c]
Origanum vulgare	12.4 ± 2.7	14.0 ± 2.7
Origanum majorana L.	2.2 ± 0.4	2.5 ± 0.5
Salvia officinalis L.	1.0 ± 0.6	1.1 ± 0.6
Levisticum	1.3 ± 0.6	1.4 ± 0.7

API contents in oregano (14.0 mg/g of dry sample) and marjoram (2.5 mg/g of dry sample) were also high, and the results obtained are comparable to those described earlier [31, 32].



https://www.themossreport.com/parsley-component-fights-cancer/
These Ohio scientists found that apigenin effectively inhibited a molecule called IKKα. IKKα is an enzyme complex involved in regulating a transcription factor called NF-kappaB, responsible for cellular response to inflammation (Häcker 2006). They describe IKKα as a "key driver of the metastatic process" and therefore a "promising therapeutic target in anticancer drug research."
A small but interesting clinical trial was performed in Groß-Gerau, Germany, and was published by Prof. Harald Hoensch of the University of Frankfurt. His group gave a food supplement of 10 milligrams (mg) of apigenin as well as 10 mg of EGCg (a main ingredient in green tea) to patients who had either colorectal cancer or premalignant polyps of the colon. The results were dramatic. In the control group, 47 percent (7 out of 15) had recurrences either of cancer or of their polyps. But in the treated group, only 7 percent (1 out of 14) had a recurrence. Writing in the World Journal of Gastroenterology. Hoensch said:
"Sustained long-term treatment with a flavonoid mixture could reduce the recurrence rate of colon neoplasia [cancer, ed.] in patients with resected colon cancer" (Hoensch 2008).
The most abundant sources are dried parsley leaves and grapefruit. According to one nutritional Web site (merschat.com), dried parsley has an incredible **13,000 mg per 100 grams.**

https://www.researchgate.net/publication/7363831_Bioavailability_of_Apigenin_from_Apiin-Rich_Parsley_in_Humans
Bioavailability of Apigenin from Apiin-Rich Parsley in Humans 2006
After an apigenin- and luteolin-free diet, a single oral bolus of 2 g blanched parsley (corresponding to 65.8 +/- 15.5 micromol apigenin) per kilogram body weight was consumed. Blood samples were taken at 0, 4, 6, 7, 8, 9, 10, 11 and 28 h after parsley consumption and 24-hour urine samples were collected. Apigenin was analyzed in plasma, urine and red blood cells by means of HPLC-ECD. On average, a maximum apigenin plasma concentration of 127 +/- 81 nmol/l was reached after 7.2 +/- 1.3 h with a high range of variation between subjects. For all participants, plasma apigenin concentration rose after bolus ingestion and fell within 28 h under the detection limit (2.3 nmol/l). The average apigenin content in 24-hour urine was 144 +/- 110 nmol/24 h corresponding to 0.22 +/- 0.16% of the ingested dose.

https://www.tandfonline.com/doi/full/10.1080/10942912.2023.2236329#abstract
Therapeutical properties of apigenin: a review on the experimental evidence and basic mechanisms 2023
The apigenin contents were reported high in celery and parsley with amounts of 19 and 215 mg per 100 g, respectively.[Citation1] Other sources rich in this bioactive molecule include wheat sprouts, oranges, rutabagas, tea, onion, chamomile, and cilantro.
According to reports, dried parsley contains highest concentration of apigenin (45,035 µg/g). The dried chamomile flowers contain 3,000 to 5,000 µg/g of apigenin. Taken together, apigenin alone or in combination with other inhibitors such as curcumin, sorafenib, or gefitinib has the potential to manage highly aggressive cancers. In concusion, apigenin has showed promising *in vitro* and *in vivo* anticancer properties that warrants further investigation *via* clinical studies.

https://www.cambridge.org/core/journals/british-journal-of-nutrition/article/flavone-apigenin-blocks-nuclear-translocation-of-sterol-regulatory-elementbinding-protein2-in-the-hepatic-cells-wrl68/2813A579F8F7C323C208F064DC6D8A63
The flavone apigenin blocks nuclear translocation of sterol regulatory element-binding protein-2 in the hepatic cells WRL-68 2015
Sterol regulatory element-binding protein-2 (SREBP-2) is a pivotal transcriptional factor in cholesterol metabolism. Factors interfering with the proper functioning of SREBP-2 potentially alter plasma lipid concentrations. Consuming fruits and vegetables is associated with beneficial plasma lipid profile. The mechanism by which plant foods induce desirable lipid changes remains unclear. Apigenin, a common plant food flavonoid, was shown to modulate the nuclear translocation of SREBP-2 in the hepatic cells WRL-68 in the present study.

In summary, the present study showed that apigenin prevented SREBP-2 translocation and reduced the downstream gene HMGCR transcription. The minimum effective dosage should be achievable in the form of functional food consumption or dietary supplementation.
The daily intake of apigenin in another study is about 4 mg, the plasma concentration is roughly 0·01 μm, and the maximum plasma concentration can be as high as 0·4 μm at 8 h after celery leaf consumption(Reference Cao, Zhang and Chen 33). Going by the data of the present study, the effective dose of 1 μm should fall within the functional food or dietary supplement range of consumption in human beings. According to a pharmacokinetic study in rats(Reference Chen, Tu and Sun 34), oral dosages of 5·4 mg apigenin/kg body weight would produce a C_{max} value of 16·5 μm in serum.
Besides, apigenin is widely found in traditional herbal medicine. Given its high bioavailability, its action on cholesterol synthesis could be achievable in this administrative method. However, the final drug concentration still relies on the specific herbal composition of prescription.
Statins have been prescribed clinically for controlling blood cholesterol, and they inhibit HMGCR activity. Apigenin, by contrast, might act on the enzyme's upstream transcriptional factor. In conclusion, the present study renewed our understanding of apigenin–SREBP-2 interaction at the post-translational level. These findings also supported the hypothesis that consuming apigenin-rich food may prevent hypercholesterolemia.

https://www.researchgate.net/publication/363656562_The_Potential_Role_of_Apigenin_in_Cancer_Prevention_and_Treatment

The Potential Role of Apigenin in Cancer Prevention and Treatment 2022

Flavonoids have beenconsidered to have scientific interest, as they show numerous pharmalogical activities such as anti-oxidant activity, anti-inflammation, free radical scavenging, and anti-cancer activity, regulating cellular proliferation, induction of apoptosis, preventing platelet aggregation as well as reducing plasma levels of low-density lipoproteins [6–9]. Among the over 6000 different flavonoids, **apigenin**, **myricetin quercetin**, **luteolin** and **kaempferol** are the five most ubiquitous plant flavonoids [10] (Figure 1). Apigenin (APG) is a consumable flavonoid (4′,5,7-trihydroxyflavone) that has been become popularized as a health-promoting drug in recent years owing to its poor intrinsic toxicity and distinct activities on
normal versus cancer, compared with other structurally related flavonoids [11].

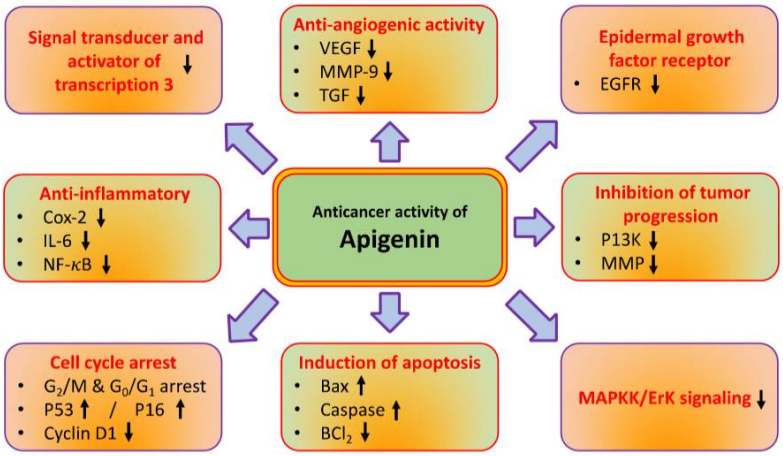


Figure 2. Apigenin's role in cancer management through modulating cell signaling pathways. The complex cell nature of cancer is characterised by a number of complex molecular interactions and mechanisms.

Table 1. Major mechanism of apigenin in cancer management through modulating gene activity.

Genes	Mechanism	Refs.
TNFα	Apigenin downregulates the TNFα mediated release of chemokines and suppresses Interlukin-6 and interlukin-1α.	[16]
NF-κB	Apigenin can regulate the production as well as the gene expression of mucin via regulating the nuclear factor-κB signaling pathway in airway epithelial cells.	[17]
P53	The higher detection of Bax was related to greater p53 accumulation. It suggested that apigenin boosted the cytotoxic impact of cisplatin by inducing p53 accumulation and p53-regulated proapoptotic gene expression.	[18]
Bcl-2 and Bax	Apigenin was shown to be associated with a decrease in Bcl-xL and Bcl-2 levels, as well as an increase in the active form of the Bax protein.	[19]
Bax/Bcl-2	PARP cleavage DNA fragmentation revealed that apoptosis was induced by apigenin treatment. These effects linked to a rise in the Bax/Bcl-2 ratio, which indicates a change favoring apoptosis.	[20]
VEGF	Apigenin inhibited HIF-1alpha and vascular endothelial growth factor expression in the tumor tissues, showing an inhibitory effect of apigenin on angiogenesis.	[21]
VEGF	Apigenin demonstrated that it had a role in the inhibition of the hypoxia-induced expression of vascular endothelial growth factor mRNA	[22]
P53	Apigenin exposure induces G2/M phase cell cycle arrest, DNA damage, apoptosis and p53 accumulation, which collectively suppressing cancer cell proliferation in vitro and in vivo.	[23]
Cyclin B, cyclin A, and cyclin-dependent kinase-1	Apigenin inhibited the expression of cyclin B, cyclin A, and cyclin-dependent kinase-1, all of which are involved in the cell cycle G2-to-M transition.	[24]
PI3K/Akt/mTOR pathway	Apigenin plays a role in the induction of apoptosis as well as autophagy via the inhibition of the pathway of PI3K/Akt/mTOR	[25]
PI3K/Akt/FoxO-signaling pathway	Apigenin inhibited prostate tumorigenesis in transgenic prostate adenocarcinoma via the FoxO/PI3K/Akt signaling cascade.	[26]
IKKα	Apigenin straight binds with IKKα, decreases IKKα kinase activity, and subdues NF-κB/p65 activation in cancer cells much more effectively than than an IKK inhibitor	[27]
ERK	TRAIL-induced antitumor activity in lung cancer cells by the treatment of apigenin through inhibition of the ERK, Nuclear factor F-κB, and AKT prosurvival regulators	[28]
ERK	Apigenin suppressed AKT and ERK activation. Moreover, it enhanced ABT-263-induced antitumor activity in colon cancer cells via apigenin through the inhibition of the AKT, Mcl-1 as well as ERK prosurvival regulators	[29]
ERK	Phosphorylation of AKT, P70RSK, and S6 was decreased by apigenin while the phosphorylation of ERK1/2 and P90RSK was increased by apigenin treatment	[30]
STAT3	Apigenin activated p53 that induced catalase, a reactive oxygen species scavenger enzyme, and inhibited Signal transducer and activator of transcription 3, the most important pro-survival pathway in primary effusion lymphoma.	[31]
STAT3	apigenin efficiently repressed Signal transducer and activator of transcription 3phosphorylation, decreased STAT3 nuclear localization and repressed Signal transducer and activator of transcription 3transcriptional activity	[32]
MMP	Apigenin down-regulated Signal transducer and activator of transcription 3target genes MMP-2, MMP-9 and vascular endothelial growth factor that participate in cell migration and invasion	[32]
EFGR	Apigenin and Cetuximab could decrease the expressions of p- epidermal growth factor receptor, p-Akt, p-Signal transducer and activator of transcription 3 and Cyclin D1	[33]
Wnt/β-catenin signaling pathway	Apigenin significantly suppressed colorectal cancer cell proliferation, migration, invasion and organoid growth through inhibiting the Wnt/β-catenin signaling pathway	

https://www.researchgate.net/publication/361479151_Apigenin_in_Cancer_Prevention_and_Therapy_A_Systematic_Review_and_Meta-Analysis_of_Animal_Models

Apigenin in Cancer Prevention and Therapy: A Systematic Review and Meta-Analysis of Animal Models 2022

Overall, Apigenin reduces tumor volume (SMD=-3.597, 95% CI: -4.502 to -2.691, p<0.001), tumor-weight (SMD=-2.213, 95% CI: -2.897 to -1.529, p<0.001), tumor number (SMD=-1.081, 95% CI: -1.599 to -0.563, p<0.001) and tumor load (SMD=-1.556, 95% CI: -2.336 to -0.776, p<0.001). Further, it has no significant effect on the animal's body-weight (SMD=-0.345, 95% CI: -0.832 to 0.143, p=0.165). Apigenin exerts anti-tumor effects mainly by inducing apoptosis/cell-cycle arrest. Conclusions Our analysis suggests that Apigenin has potential anticancer effects against various cancers. However, the poor symmetry of the funnel plot suggested publication bias. Thus, it warrants further research to evaluate the potential of Apigenin alone or as an adjuvant for cancer treatment.

https://pmc.ncbi.nlm.nih.gov/articles/PMC11122459/

Apigenin: Molecular Mechanisms and Therapeutic Potential against Cancer Spreading 2024

It is well documented that Apigenin (4′,5,7-trihydroxyflavone), among other flavonoids, is able to modulate key signaling molecules involved in the initiation of cancer cell proliferation, invasion, and metastasis, including JAK/STAT, PI3K/Akt/mTOR, MAPK/ERK, NF-κB, and Wnt/β-catenin pathways, as well as the oncogenic non-coding RNA network. Based on these premises, the aim of this review is to emphasize some of the key events through which Apigenin suppresses cancer proliferation, focusing specifically on its ability to target key molecular pathways involved in angiogenesis, epithelial-to-mesenchymal transition (EMT), maintenance of cancer stem cells (CSCs), cell cycle arrest, and cancer cell death.

Food sources containing the highest amounts of Apigenin.

Source	Concentration (µg/g)
Dried parsley	45,035
Dried chamomille flower	3000–5000
Parsley	2154.6
Celery seed	786.5
Vinespinach	622
Chinese celery	240.2
Kumquats	218.7
Celery	191
Dried oregano	177.1
Artichoke	74.8
Juniper berries	72.6
Peppermint	53.9

Apigenin (Apigenin-7-O-glucoside), Vitexin (Apigenin-8-C-D-glucopyranoside), isovitexin (Apigenin-6-C-glucoside), rhoifolin (Apigenin-7-O-neohesperidoside), and schaftoside (Apigenin-6-C-glucoside-8-C-arabinoside) represent the more abundant Apigenin glycosides in nature [18].

The production of ROS is generally increased in cancer cells due to their very high metabolic rates and the hypoxic conditions that support the rapid and massive growth of tumor cells [159]. Depending on the physio-pathological context, flavonoids can act both as pro- and antioxidant messengers [11]. The anticancer **activity of Apigenin has been linked to the induction of oxidative stress in cancer cells** and the promotion of apoptotic cell death [114]. Furthermore, Apigenin induced apoptosis in human breast, cervical, melanoma, lung, prostate, and head and neck cancer cells [121,138,145,160,161,162,163,164], triggering intracellular ROS accumulation and loss of mitochondrial integrity, as proved by low MMP in Apigenin-treated cells [138,158,163].

Lowering the cell's antioxidant defense system is another mechanism through which Apigenin increases oxidative stress [9,114]. This has been demonstrated in hepatocellular cancer cells, where catalase and glutathione (GSH), molecules involved in alleviating oxidative stress, were downregulated after Apigenin administration [165].

Moreover, crosstalk between p53 activation and the STAT3 pathway has been studied recently. For example, in lymphoma cells, Apigenin promoted p53 activation, which mediated ROS reduction through catalase induction and inhibited the prosurvival pathway of STAT3, which has an inhibitory action on p53 [180].

However, despite the multiple anti-cancer therapeutic properties of this compound, its biological applications are limited by its hydrophobic nature and consequently, its bioavailability. This last aspect limits its clinical use; thus, to improve the bioavailability of this compound, several alternatives are being developed for new formulations, including nanoparticles and similar devices [260].

https://pmc.ncbi.nlm.nih.gov/articles/PMC10630840/

A comprehensive view on the apigenin impact on colorectal cancer: Focusing on cellular and molecular mechanisms 2023

The results of this review study show that apigenin has potential anticancer effects on CC cells through various mechanisms. In this comprehensive review, we present the cellular targets and signaling pathways of apigenin indicated to date in in vivo and in vitro CC models. Among the most important modulated pathways, Wnt/β-catenin, PI3K/AKT/mTOR, MAPK/ERK, JNK, STAT3, Bcl-xL and Mcl-1, PKM2, and NF-kB have been described. Furthermore, apigenin suppresses the cell cycle in G2/M phase in CC cells. In CC cells, apigenin-induced apoptosis is increased by inhibiting the formation of autophagy. According to the results of this study, apigenin appears to have the potential to be a promising agent for CC therapy, but more research is required in the field of pharmacology and pharmacokinetics to establish the apigenin effects and its dosage for clinical studies.

Treating patients with apigenin was found to increase p53 and p21CIP1/WAF1 (Lee et al., 2014). Apigenin also enhanced cellular cycling and cell suicide by hindering the PI3K/Akt/mTOR pathway (Yang et al., 2018). Scientists have discovered that apigenin has the prospect of preventing cancer by controlling the ERK1/2 MAPK and PI3K/Akt signaling pathways, thus stopping the growth and spread of tumors (Lim et al., 2016). In addition, by inhibiting the Wnt/β-catenin pathway, apigenin significantly reduced the growth, and spread of malignant cells, their ability to divide, invade surrounding tissues, migrate to new areas, and form organoids (Xu et al., 2016).

Hydrophobic compounds generally exhibit poor bioavailability because of limited capability of absorption, resulting in low levels of the drug reaching the target tumor and advanced toxicity to normal tissues (Darakhshan et al., 2015).

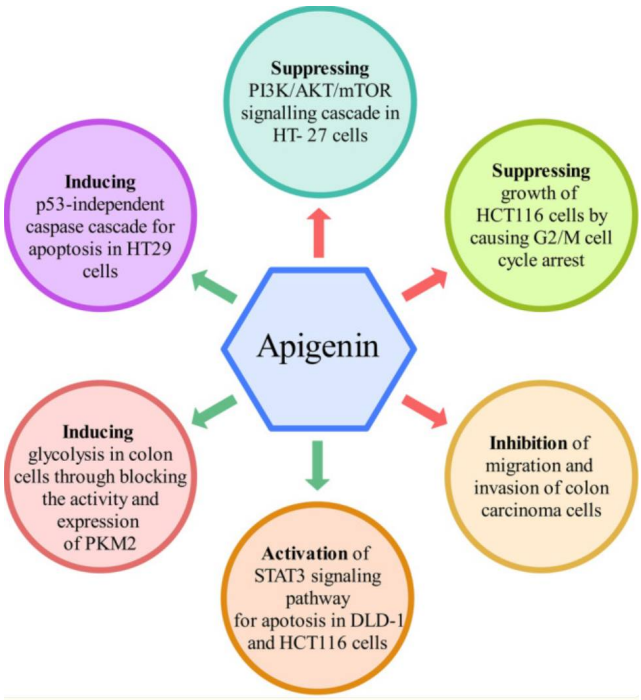
Apigenin is not easily absorbed orally because of its low water solubility, which is only 2.16 g/mL (Salehi et al., 2019), significantly hindering its clinical development.

Apigenin is slowly absorbed and eliminated from the body, as evidenced by its half-life of 91.8 h in the blood ...

Apigenin, a plant-derived compound, has demonstrated selective anticancer effects and effective cell cytotoxic activity while exhibiting negligible toxicity to ordinary cells. It has also been found to quash cancer stem cells (CSCs; Ketkaew et al., 2017).

Apigenin is generally considered safe for intentional consumption in higher doses, as the toxicity hazard is low. Apigenin quantity in a person's diet is not likely to get to a level that would cause injury (Shao et al., 2013).

Apigenin has been reported to suppress CC in vitro and in vivo by multiple biological effects, such as triggering cell apoptosis and autophagy, inducing cell cycle arrest, and suppressing cell migration and invasion. We summarized the effects of apigenin on CC cells in Figure 4. Taken together, these results indicate that apigenin could inhibit the growth of CC cells in vitro and in vivo and may be used for the improvement of therapy for colon cancer.



https://cancerci.biomedcentral.com/articles/10.1186/s12935-021-02282-3

Chemoprotective and chemosensitizing effects of apigenin on cancer therapy 2021

Conclusion

Briefly, our analysis proposed that the combination therapies with apigenin could suppress the unwanted toxicity of chemotherapeutic agents. It is believed that these expedient results may pave the path for the development of drugs with a high therapeutic index. Nevertheless, human clinical trials are a prerequisite to consider the potential use of apigenin in the prevention and treatment of various cancers. Conclusively, the clinical trials to comprehend the role of apigenin as a chemoprotective agent are still in infancy.

Flavonoids are assumed to be safe nutritionally, while apigenin implicates low toxicity [31]. However, evaluation of the acute toxicity of apigenin resulted in no mortality or signs of toxicity in mice/rats at oral doses up to 5000 mg/kg [32]. Moreover, in vitro evaluation of carcinogenicity proved that apigenin has no toxic or mutagenic effects [33, 34]. Intriguingly, after 30 min treatment in vitro, the hemolytic activity of apigenin was reported to be lower than the acceptable limit of 5% signifying its potential safety in intravenous dosages [35].

https://pubmed.ncbi.nlm.nih.gov/27939619/

Formulation and characterization of an apigenin-phospholipid phytosome (APLC) for improved solubility, in vivo bioavailability, and antioxidant potential 2017

The apigenin-phospholipid phytosome (APLC) was developed to improve the aqueous solubility, dissolution, in vivo bioavailability, and antioxidant activity of apigenin.

The optimized formulation demonstrated over 36-fold higher aqueous solubility of apigenin, compared to that of pure apigenin. The formulation also exhibited a significantly higher rate and extent of apigenin release in dissolution studies.

The prepared formulation also exhibited antioxidant potential by significantly **increasing the levels of glutathione**, superoxide dismutase, catalase, and decreasing the levels of lipid peroxidase. The study shows that phospholipid-based phytosome is a promising and viable strategy for improving the delivery of apigenin and similar phytoconstituents with low aqueous solubility.

https://www.researchgate.net/publication/359907593_Chemotherapeutic_effects_of_Apigenin_in_breast_cancer_Preclinical_evidence_and_molecular_mechanisms_enhanced_bioavailability_by_nanoparticles

Chemotherapeutic effects of Apigenin in breast cancer: Preclinical evidence and molecular mechanisms; enhanced bioavailability by nanoparticles 2022

However, AP is categorized as a Class II drug of Biopharmaceutical Classification System with low solubility in water which limited its therapeutic effects. Therefore, nanotechnology due to the presentation of remarkable properties has overcome this limitation through enhanced the solubility and bioavailability of AP. In this regard, various nanocarriers such as nanocrystals, micelles, liposomes, PLGA, etc., have highlighted the significantly increased bioavailability and therapeutic efficacy of AP.

. Increase bioavailability of AP Based on the literature, there are several methods to increase the bioavailability of AP in different forms and concentrations, for different types of applications. Based on the biopharmaceutics classification systems, AP is a class II drug with considerable intestinal membrane permeability and also has very poor solubility in green media, especially water [70, 71]. There is wide interest in improving the bioavailability of AP via cross-linking them with biocompatible linkers, and also modifying the chemical structure with reactive functional groups; however, the most important factor in the improving of AP bioavailability, generally any compound, is considering the factors that may play a role in the mass production or entry of these compounds into the clinical phase [72, 73]. In this manner, Rabiee's Theory (Variable Laws) [74] would be considered as a promising point.

https://www.researchgate.net/publication/42373076_Apigenin_A_Promising_Molecule_for_Cancer_Prevention

Apigenin: A Promising Molecule for Cancer Prevention 2010

Epidemiologic studies considerably support the notion that diets rich in plant flavones are associated with a number of health benefits, including a reduction of the risk of developing certain cancers. Integration of dietary modification rich in flavones might be a comprehensive chemopreventive strategy for the high-risk

individual that may have impact in the neoplastic transformation. Since apigenin is one of the most bioactive plant flavone and is widely distributed in common fruits, beverages and vegetables, its consumption through diet is highly recommended.

https://www.researchgate.net/publication/351818123_Apigenin_inhibits_the_growth_of_colorectal_cancer_through_down-regulation_of_E2F13_by_miRNA-215-5p

Apigenin inhibits the growth of colorectal cancer through down-regulation of E2F1/3 by miRNA-215-5p 2021

Cell proliferation and apoptosis of human colon cancer cell line HCT116 was assessed after API treatment. A comprehensive transcriptome profile of API -treated HCT116 cells was acquired by high-throughput sequencing. The regulation of miRNA215-5p and E2F1/3 were identified by bioinformatics analyses. An inhibitor of miRNA215-5p, inhibitor 215, was applied to confirm the role of this microRNA played in the anti-cancer effect of API. Luciferase reporter gene assay was performed to identify targeting relationship between miRNA215-5p and E2F1/3. Result : API significantly promoted cell apoptosis and anti-proliferation of HCT116 cells in a dose-dependent manner. Bioinformatics analyses identified several altered miRNAs among which the expression of miRNA-215-5p showed markedly increased. Meanwhile, the expression of E2F1 and E2F3 was decreased by API, which was associated with miRNA215-5p. Luciferase reporter gene assay showed miRNA-215-5p could directly bind to 3' UTR of E2F1/3. Inhibition of miRNA-215-5p significantly inhibited apoptosis and cell cycle arrest at G0/G1 phase induced by API. Conclusions : The result of this study confirmed the anti-cancer effect of API on human colorectal cancer cells and investigated the underlying mechanism by a comprehensive transcriptome profile of API-treated cells.

https://pmc.ncbi.nlm.nih.gov/articles/PMC5207605/

Role of Apigenin in Cancer Prevention via the Induction of Apoptosis and Autophagy 2016

The chemopreventive effect of apigenin was explored in at least dozen in vivo studies, which tested doses, administration routes, and treatment frequencies of apigenin. The oral administration of apigenin (20 and 50 μg/mice) for 20 weeks reduced tumor volumes and induced complete abolishment of distant organ metastasis in the transgenic adenocarcinoma of a mouse prostate (TRAMP) model. This effect was attributed to the suppression of the phosphoinositide 3-kinase (PI3K)/Akt/Forkhead box O-signaling pathway.14 The same research group also showed that apigenin effectively suppressed prostate cancer progression in TRAMP mice by attenuation of insulin-like growth factor (IGF)-I/IGF binding protein-3 signaling and inhibition of angiogenesis and metastasis.15

In addition, a 15-week period of oral administration of apigenin (2.5 mg/kg) in hamsters resulted in reduction of tumor volume and incidence, modulation of cell proliferation, apoptosis, inflammation, and angiogenesis markers, and modulation of phase I and II detoxification cascades in a 7,12-dimethyl benz[*a*]anthracene (DMBA)-induced experimental oral carcinogenesis model.16,17

A study by Byun et al.21 showed reduction of UVB-induced ear edema and inflammatory mediator COX-2 expression in the skin of SKH-1 hairless mouse, reflecting the potent chemopreventive activity of apigenin against UVB-induced skin inflammation. A topical application of apigenin (5 μM) prior to UVB-exposure attenuated the expression of COX-2 and hypoxia inducible factor (HIF)-1α, important mediators of angiogenesis, through modulation of HuR and thrombospondin-1.22 Another study showed that apigenin inhibited activation of the UVB-induced mammalian target of rapamycin (mTOR), cell proliferation, and cell cycle progression in mouse skin. The same study also demonstrated that apigenin inhibited UVB-induced mTOR signaling mainly through the activation of AMP-activated protein kinase (AMPK), rather than the suppression of Akt, even though UVB-induced mTOR activation is driven by PI3K/Akt signaling and apigenin is capable of blocking Akt phosphorylation/activation.23

https://pmc.ncbi.nlm.nih.gov/articles/PMC6817918/

A Review on Flavonoid Apigenin: Dietary Intake, ADME, Antimicrobial Effects, and Interactions with Human Gut Microbiota 2019

Dried parsley has a particularly high level of apigenin that far exceeds any other vegetables or herbs [5]. Chamomile tea, high in apigenin content, is one of the most common sources of apigenin intake from a single ingredient [19].

Apigenin is the aglycone form and it is present in plants naturally as several apigenin glycosides as mentioned in the previous section. Those glycoside conjugates, primarily as apigenin-7-*O*-glucoside, and acylated derivatives are more water soluble than apigenin [10] and their structures have a major impact on their absorption and bioavailability, with the best bioavailability occurring when apigenin is bound to *β*-glycosides [9].

Apigenin is practically insoluble in highly polar solvents such as water (0.00135 mg/mL), and nonpolar solvents such as silicon fluid (0.0728 mg/mL) and safflower oil (0.0317 mg/mL) [15, 16]. Other reports on apigenin's solubility in aqueous solutions show that it ranges from 0.001 to 1.63 mg/mL in nonpolar solvents [6] and 2.16 *μ*g/mL in phosphate buffer at pH 7.5 [31]. Apigenin is freely soluble in dimethylsulfoxide (DMSO) [11]. One source estimated the solubility to be more than 100 mg/mL [16], while another showed that the approximate solubility of apigenin in ethanol, DMSO, and dimethylformamide (DMF) purged with inert gas to be 0.3, 15, and 25 mg/mL [32]. Flavonoids are also more soluble in methanol than in water [33]. As a result, **organic solvents like DMSO [34] and Tween 80 [31]** are used to dissolve apigenin prior to their addition to an aqueous solution to increase solubility. Different carriers such as ethosomes [35], polymeric micelles of Pluronic P123 and Solutol HS 15 [36], and carbon nanopowder [37], or self-microemulsifying delivery system [38] are also developed and tested to enhance the efficacy of apigenin.

https://www.ars.usda.gov/ARSUserFiles/80400535/Data/Flav/Flav3.3.pdf

USDA Database for the Flavonoid Content of Selected Foods, Release 3.3 (2018)

02029 Spices, parsley, dried

(Petroselinum crispum)		Mean(mg/100g)	Min	Max
Flavones	Apigenin	4503.50	1774.60	13506.22
	Luteolin	19.75	19.75	19.75
Flavonols	Isorhamnetin	331.24	331.24	331.24
	Kaempferol	0.00	0.00	0.00
	Quercetin	0.00	0.00	0.00 C

https://www.nature.com/articles/s41598-024-59617-z

Dissolution and antioxidant potential of apigenin self nanoemulsifying drug delivery system (SNEDDS) for oral delivery 2024

Self-nanoemulsifying drug delivery systems (SNEDDS) have been used to improve the oral bioavailability of various drugs. In the current study, apigenin was developed as SNEDDS to solve its dissolution problem and enhance oral bioavailability and antioxidant potential. SNEDDS were prepared by mixing Gelucire 44/14, Tween 80, and PEG 400 under controlled conditions. The droplet of diluted SNEDDS demonstrated a spherical shape with a size of less than 100 nm and a neutral charge

The chosen formulations had a specific Smix ratio of 1:1 and concentrations of Gelucire 44/14, Tween 80, and PEG 400 in the ranges of 5–40% w/w, 30–47.5% w/w, and 30–47.5% w/w, respectively, as shown in Table 1. The apigenin-free SNEDDS (blank) was prepared by mixing Gelucire 44/14 with Tween 80:PEG 400 (Smix) according to the formula ratio. For apigenin-loaded SNEDDS, 0.5% w/w of apigenin was added to the blank SNEDDS and continuously stirred at 40 °C for 30 min. The excess undissolved apigenin was separated to obtain a clear mixture.

https://www.sciencedirect.com/science/article/abs/pii/S0928098713000055
Preparation of apigenin nanocrystals using supercritical antisolvent process for dissolution and bioavailability enhancement 2013
The prepared AP nanocrystals, without change in crystalline structure, appeared in spherical shape with particle size of about 400–800 nm. The reduction of particle size resulted in a more rapid dissolution of AP from nanocrystals than from coarse powder. In comparison to coarse powder, AP nanocrystals exhibited a significantly decreased t_{max}, a 3.6-fold higher peak plasma concentration (C_{max}) and 3.4-fold higher area under the curve (*AUC*).

https://pmc.ncbi.nlm.nih.gov/articles/PMC8241174/
Preparation, characterization and antitumor activity evaluation of apigenin nanoparticles by the liquid antisolvent precipitation technique 2017
The present work aimed to apply the liquid antisolvent precipitation (LAP) method for preparing the apigenin nanoparticles and thereby improving the solubility and bioavailability of apigenin.
Under the optimum conditions, the particle size of the apigenin nanosuspension was about 159.2 nm. In order to get apigenin nanoparticles, the freeze-drying method was selected and the mannitol was used as a cryoprotectant.
The oral bioavailability of apigenin nanoparticles was about 4.96 times higher than that of the raw apigenin, but the apigenin nanoparticles had no toxic effect on the organs of rats. In addition, the apigenin nanoparticles had a higher inhibition to HepG2 cells by lower IC50 than that of raw apigenin.
In addition, The IC50 values of apigenin nanoparticles and raw apigenin were separately 89.33 and 216.84 μg/mL, it was also seen that the apigenin nanoparticles had a higher inhibition to HepG2 cells by lower IC50 than that of raw apigenin.

https://pmc.ncbi.nlm.nih.gov/articles/PMC10680483/
Dually Active Apigenin-Loaded Nanostructured Lipid Carriers for Cancer Treatment 2023
The production of APG-NLC was carried out using the hot high-pressure homogenisation method (Homogeniser FPG 12800, Stansted, United Kingdom). Firstly, a primary emulsion with a mixture of an aqueous phase containing the surfactant, and a lipid phase containing the lipids and the drug, was prepared using an Ultraturrax® T10 basic (IKA, Germany) at 8.000 rpm for 30 s. The production conditions were 85°C, three homogenisation cycles, and 900 bar of pressure.[28]
Moreover, the IC_{50} of the APG-NLC was 10–27 times lower against cancer cells than that of free APG.
Moreover, the comparison of APG-NLC anticancer activity against two different breast cancer models showed that APG-NLC after 24 h and 72 h was more active against triple negative breast cancer (TNBC) MDA-MB-468 cells (IC_{50} 2.9 μg/mL and 0.41 μg/mL) than ER-positive MCF-7 cells (IC_{50} 16.2 μg/mL and 1.8 μg/mL).
On the one hand, the non-ionic surfactant Tween 80® can effectively inhibit P-gp and plays an important role in mediating the opening of tight junctions, which could enhance the paracellular uptake of NLC, increasing their permeability.[86,87]

https://pmc.ncbi.nlm.nih.gov/articles/PMC7683501/
Apigenin-Loaded Solid Lipid Nanoparticle Attenuates Diabetic Nephropathy Induced by Streptozotocin Nicotinamide Through Nrf2/HO-1/NF-kB Signalling Pathway 2020
Preparation of Apigenin-SLNPs
We used microemulsification method to prepare SLNPs. We then briefly placed a mixture of 45.45% Tween 80, 0.58% PLPC, and water in a beaker which was then subjected to heat to a temperature to attain the lipid-melting point. We also melted Lipid (7.27%) at a temperature of 82°C to 85°C separately. We then added Apigenin (25 mg) aqueou phase containing Tween 80, later we dropped the hot aqueousemulsifier mix into the lipid melt all at once. This was done under magnetic stirring for obtaining a clear micro-emulsion. We then transferred hot microemulsion in cold water (~2°C) of an equal amount. This process was carried out under mechanical stirring at a speed of 5000 rpm for a time period of 1.5 hrs. The SLNPs are formed in aqueous medium by crystallization of the hot droplets of lipid that are present in the microemulsion. The aqueous SLNP dispersion that was prepared was refrigerated until further analysis.13

https://www.sciencedirect.com/science/article/abs/pii/S0022354916326570
Polysorbates 20 and 80 Used in the Formulation of Protein Biotherapeutics: Structure and Degradation Pathways 2008
Polysorbates 20 and 80 (Tween® 20 and Tween® 80) are used in the formulation of biotherapeutic products for both preventing surface adsorption and as stabilizers against protein aggregation. The polysorbates are amphipathic, nonionic surfactants composed of fatty acid esters of polyoxyethylene sorbitan being polyoxyethylene sorbitan monolaurate for polysorbate 20 and polyoxyethylene sorbitan monooleate for polysorbate 80. The polysorbates used in the formulation of biopharmaceuticals are mixtures of different fatty acid esters with the monolaurate fraction of polysorbate 20 making up only 40-60% of the mixture and the monooleate fraction of polysorbate 80 making up >58% of the mixture.
Polysorbate 20 (polyoxyethylene sorbitan monolaurate) and Polysorbate 80 (polyoxyethylene sorbitan monooleate) are the most common polysorbates currently used in formulation of protein biopharmaceuticals.[22]
Polysorbates are present in a large number of biopharmaceutical drugs listed in the 2006 Physicians Desk Reference. The concentrations used in the formulations range from 0.0003% (w/v) to 0.3% (w/v).

https://pmc.ncbi.nlm.nih.gov/articles/PMC7022221/
Pediatric Safety of Polysorbates in Drug Formulations 2019
The FDA has granted PS80 the status of generally recognized as safe (GRAS), and both PSs are listed in its Inactive Ingredients Database [8]. Per the WHO, the accepted oral daily intake of PSs by adults is 25 mg/kg, compared with 10 mg/kg as suggested by the Scientific Committee on Food [9,10].

https://pubmed.ncbi.nlm.nih.gov/32059077/
Apigenin as an anticancer agent 2020
Apigenin is an edible plant-derived flavonoid that has been reported as an anticancer agent in several experimental and biological studies. It exhibits cell growth arrest and apoptosis in different types of tumors such as breast, lung, liver, skin, blood, colon, prostate, pancreatic, cervical, oral, and stomach, by modulating several signaling pathways. Apigenin induces apoptosis by the activation of extrinsic caspase-dependent pathway by upregulating the mRNA expressions of caspase-3, caspase-8, and TNF-α. It induces intrinsic apoptosis pathway as evidenced by the induction of cytochrome c, Bax, and caspase-3, while caspase-8, TNF-α, and B-cell lymphoma 2 levels remained unchanged in human prostate cancer PC-3 cells. Apigenin treatment leads to significant downregulation of matrix metallopeptidases-2, -9, Snail, and Slug, suppressing invasion. The expressions of NF-κB p105/p50, PI3K, Akt, and the phosphorylation of p-Akt decreases after treatment with apigenin. However, apigenin-mediated treatment significantly reduces pluripotency marker Oct3/4 protein expression which might be associated with the downregulation of PI3K/Akt/NF-κB signaling.

https://pubmed.ncbi.nlm.nih.gov/33333052/
Rationalizing the therapeutic potential of apigenin against cancer 2021
Key findings: The literature search resulted in many in vitro, in vivo and a few cohort studies that evidenced the effectiveness of apigenin and its analogs in modulating important molecular targets and signaling pathways such as PI3K/AKT/mTOR, JAK/STAT, NF-κB, MAPK/ERK, Wnt/β-catenin, etc., which play a

crucial role in the development and progression of cancer. In addition, apigenin was also shown to inhibit chemoresistance and radioresistance and make cancer cells sensitive to these agents. Reports have further revealed the safety of the compound and the adaptation of nanotechnological approaches for improving its bioavailability.

https://biosignaling.biomedcentral.com/articles/10.1186/s12964-022-00906-3

An update of Nrf2 activators and inhibitors in cancer prevention/promotion 2020

Api is a kind of flavonoids that plentifully exists in plant-derived beverages and vegetables including orange, onion, parsley, tea, wheat sprouts and chamomile [171]. According to the previous studies Api has different pharmacological properties, for example, antivirus [172], anti-inflammatory [173], anti-oxidant [174] and anticancer activity [175]. In addition, Api, because of low bioactivity and slow pharmacokinetics, effectively accumulates in cells/tissues [176, 177]. Api potently increases the transcription of Nrf2 and leads to the elevated level of phase II detoxification proteins in t-BHP (tert-Butyl hydroperoxide)-treated ARPE cells (Retinal pigment epithelium cells). It is noteworthy that the endogenous mRNA and protein expressions of Nrf2 and its downstream gene, hemeoxygenase-1 (HO-1), considerably are elevated by Api, which followed by cellular protection against oxidative condition [178,179,180]. While knock down or knock out of the Nrf2 by CRISPER/Cas9 system or specific shRNA lead to decreasing the protective effects of Api in oxidative stress conditions [178]. Frequently, it was reported that Api by CpG site demethylation along with attenuated activities of DNA methyltransferase and histone deacetylases capable to restore the silenced status of Nrf2 in skin epidermal JB6 P + cell line [179]. Nevertheless, in contrast with the previous study, it has been reported that Api, via down-regulating of PI3K/Akt pathway, diminishes the expression of Nrf2 at both protein and mRNA levels which leads to a reduced expression of Nrf2-target genes in BEL-7402 cells (human hepatocellular carcinoma cells). Moreover, apigenin, in combination with chrysin, directly inhibits the PI3K/Akt pathway, which is associated with the survival of cancer cells [181,182,183].

Nrf2 inhibitors

In contrast with the several agents that function as Nrf2 inducers, very few molecular components have been recognized as Nrf2 inhibitors. Since Nrf2 has multifaceted roles in cancer cells, Nrf2 inhibitors can be applied as anticancer agents [27, 77, 191]. Indirectly, Nrf2 inhibitors down-regulate drug detoxifying and eliminating enzymes and sensitize cancer cells to chemotherapeutics [76, 176]. According to the Nrf2 deactivation mechanisms and their potential applications in cancer treatment, several small molecules have been characterized as Nrf2 pathway inhibitors (Fig. 3).

Brusatol (BRU)

Brusatol is a quassinoid which is extracted from Brucea Javanica (Simaroubaceae), an evergreen shrub grown in Northern Australia and Southeast Asia [27]

Luteolin (3′,4′,5,7-tetrahydroxyflavone (LUT))

Luteolin is a natural polyphenolic flavonoid which obtained from various kinds of plants for example broccoli, celery, **parsley**, perilla leaf, and peppers, and characterized as one of the Nrf2 inhibitors [193, 196].

Trigonelline (TRG)

Trigonelline, a heterocyclic compound, is widely existing in plants, coffee and fenugreek seed that in comparison with chemicals is less toxic to humans [201].

Ascorbic acid (vitamin C, L-ascorbic acid, AscA, AA)

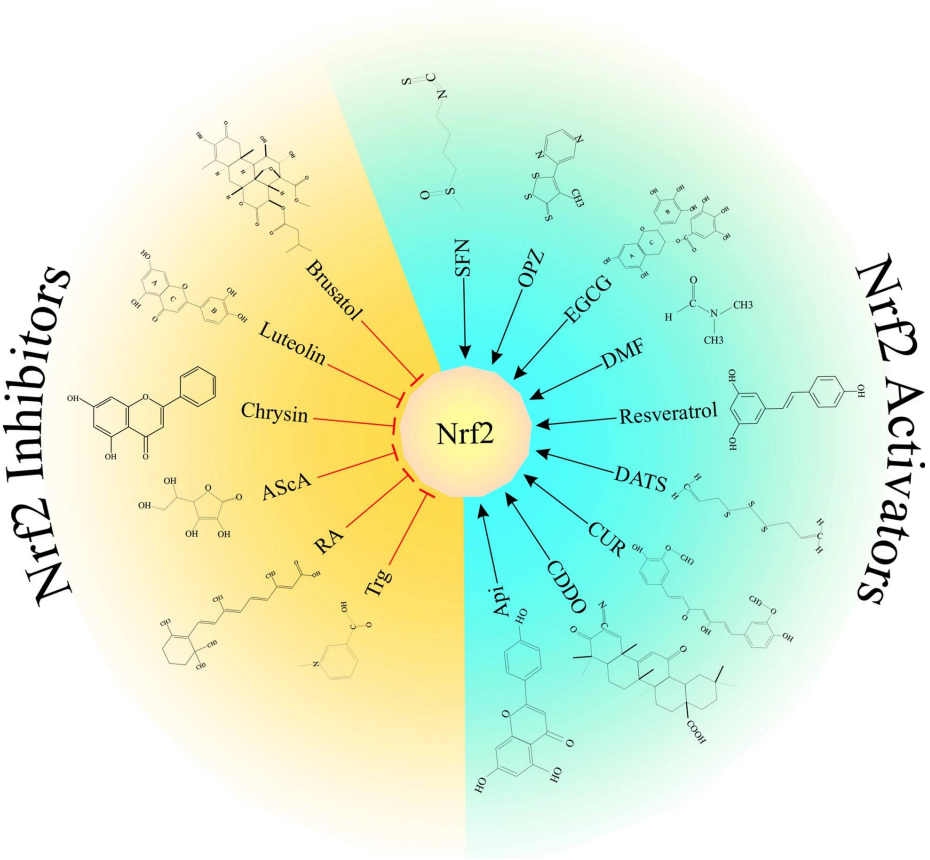
Ascorbic acid which generally known as an antioxidant agent [176], suppresses the Nrf2/DNA complex [78] and through inhibition of the nucleus translocation of Nrf2, reduces the cellular level of peroxides [176].

Retinoic acid (RA)

Retinoic Acid (RA), also known as All-trans-retinoic acid (ATRA), is a metabolite of vitamin A [207]. RA by ARE-inducing elements, for example, tBHQ, decreases the capability of Nrf2 to mediate the induction of ARE-regulated genes in both in vivo and ex vivo conditions [78].

Chrysin (5,7-dihydroxy-2-phenyl-4H-chromen-4-one (CHR))

Chrysin, a natural flavonoid, is found in many plant extracts including honey, propolis, mushroom, blue passion flower, vegetables, and fruits [212].



https://pubmed.ncbi.nlm.nih.gov/24830944/

Apigenin reactivates Nrf2 anti-oxidative stress signaling in mouse skin epidermal JB6 P + cells through epigenetics modifications 2014

API enhanced the nuclear translocation of Nrf2 and increased the mRNA and protein expression of Nrf2 and the Nrf2 downstream target gene, NQO1. Furthermore, API reduced the expression of the DNMT1, DNMT3a, and DNMT3b epigenetic proteins as well as the expression of some HDACs (1-8). Taken together, our results showed that API can restore the silenced status of Nrf2 in skin epidermal JB6 P + cells by CpG demethylation coupled with attenuated DNMT and HDAC activity. These results may provide new therapeutic insights into the prevention of skin cancer by dietary phytochemicals.

https://pubmed.ncbi.nlm.nih.gov/31953635/

Apigenin protects human melanocytes against oxidative damage by activation of the Nrf2 pathway 2020

Outcomes demonstrated that compared with negative control cultures, apigenin-treated cells exhibited enhanced viability. Likewise, apigenin enhanced expression of the cellular anti-oxidants SOD, CAT, and GSH-Px, but inhibited production of MDA, an oxidative stress biomarker. Interestingly, the expression and nuclear localization of the Nrf2 transcription factor, an important regulator oxidative stress and its downstream target genes, was significantly increased by apigenin treatment. Apigenin influence on Nrf2 was further validated by experiments demonstrating that Nrf2 knockdown cells failed to exhibit significant apigenin-mediated effects on cell viability and oxidative stress.

https://www.nature.com/articles/s41598-021-93270-0

Metformin-induced ROS upregulation as amplified by apigenin causes profound anticancer activity while sparing normal cells 2021

Metformin increased cellular ROS levels in AsPC-1 pancreatic cancer cells, with minimal effect in HDF, human primary dermal fibroblasts. Metformin reduced cellular ATP levels in HDF, but not in AsPC-1 cells. Metformin increased AMPK, p-AMPK (Thr172), FOXO3a, p-FOXO3a (Ser413), and MnSOD levels in HDF, but not in AsPC-1 cells. p-AMPK and p-FOXO3a also translocated from the cytosol to the nucleus by metformin in HDF, but not in AsPC-1 cells. Transfection of si-FOXO3a in HDF increased ROS levels, while wt-FOXO3a-transfected AsPC-1 cells decreased ROS levels. Metformin combined with apigenin increased ROS levels dramatically and decreased cell viability in various cancer cells including AsPC-1 cells, with each drug used singly having a minimal effect. Metformin/apigenin combination synergistically decreased mitochondrial membrane potential in AsPC-1 cells but to a lesser extent in HDF cells. Metformin/apigenin combination in AsPC-1 cells increased DNA damage-, apoptosis-, autophagy- and necroptosis-related factors, but not in HDF cells. Oral administration with metformin/apigenin caused dramatic blocks tumor size in AsPC-1-xenografted nude mice. Our results suggest that metformin in cancer cells differentially regulates cellular ROS levels via AMPK-FOXO3a-MnSOD pathway and combination of metformin/apigenin exerts anticancer activity through DNA damage-induced apoptosis, autophagy and necroptosis by cancer cell-specific ROS amplification.

https://pmc.ncbi.nlm.nih.gov/articles/PMC2538676/

Apigenin-induced prostate cancer cell death is initiated by reactive oxygen species and p53 activation 2009

Exposure of human prostate cancer 22Rv1 cells, harboring wild-type p53, to growth-suppressive concentrations (10–80 μM) of apigenin resulted in the stabilization of p53 by phosphorylation on critical serine sites, $p14^{ARF}$-mediated downregulation of MDM2 protein, inhibition of NF-κB/p65 transcriptional activity, and induction of p21/WAF-1 in a dose- and time-dependent manner. Apigenin at these doses resulted in ROS generation, which was accompanied by rapid glutathione depletion, disruption of mitochondrial membrane potential, cytosolic release of cytochrome *c,* and apoptosis. Interestingly, we observed accumulation of a p53 fraction to the mitochondria, which was rapid and occurred between 1 and 3 h after apigenin treatment. All these effects were significantly blocked by pretreatment of cells with the antioxidant *N*-acetylcysteine, p53 inhibitor pifithrin-α, and enzyme catalase.

https://www.ajol.info/index.php/tjpr/article/view/248901

Apigenin-7-glucoside induces apoptosis and ROS accumulation in lung cancer cells, and inhibits PI3K/Akt/mTOR pathway 2023

Purpose: To investigate how apigenin-7-glucoside (AGL) affects the proliferation, migration, invasion, and reactive oxygen species (ROS) accumulation in lung cancer cells, and to evaluate the potential of AGL as a therapeutic target.

Methods: Human bronchial epithelial cells (BEAS-2B), human lung carcinoma (A549), and non-small cell lung cancer cells (H1975), were treated with 25, 50, 100, and 200 μM AGL.

Results: Proliferation of A549 and H1975 cells was suppressed by AGL in a dose-dependent manner. AGL significantly reduced proliferation, promoted cell apoptosis, and attenuated the migration and invasion of A549 or H1975 cells. It also elevated the levels of cytochrome C and MDA but reduced the production of GSH in A549 and H1975 cells. AGL enhanced the accumulation of ROS and weakened phosphorylation of AKT, PI3K, and mTOR in A549 and H1975 cells.